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CYANONEWS

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CYANONEWS - a newsletter intended to provide cyanobacteriologists with a forum for rapid informal communication, unavailable through journals. Everything you read in this newsletter is contributed by readers like yourself. Published occasionally (about three times per year).

SUBSCRIPTION RATE - one communication every two years or so (your address label shows the date of your last communication). A communication might be a new result, news of an interesting meeting, a post-doctoral opening, a request for strains, a new article, even confirmation of your address!

WHERE TO SEND CONTRIBUTIONS - See the last page.

HOW TO GET ON THE MAILING LIST - See the last page.

HOW TO FIND OUT MORE ABOUT SOMETHING YOU READ HERE - The name of the correspondent for each item in this newsletter is capitalized, so you know who to write to for more information. The correspondent's address appears at the end of the newsletter.

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MEETINGS

It was necessary to cancel the INTERNATIONAL SYMPOSIUM ON CYANOBACTERIAL RESEARCH planned for April, owing to the loss of funding from the major symposium sponsor.

The Society for Industrial Microbiology will hold its annual meeting August 12-18, 1989, in Seattle, Washington, U.S.A. Of special interest is a symposium entitled "MICROALGAE: FROM LABORATORY TO COMMERCIAL REALITY". The lineup for the symposium is: John Benemann (Microalgae products and production: an overview), David Kyle (Production of specialty lipids), Blaine Metting (Microalgae applications in agriculture), Dan Anderson (Commercial mass culture of microalgae), and L. Brown (Applications of genetics to microalgae production). Contact Ann Kulback, SIM Headquarters, P.O. Box 12534, Arlington, VA 22209-8534, U.S.A. (Tel): 703-941-5373.

"RECENT ADVANCES IN ALGAL BIOTECHNOLOGY" is the topic of the 5th International Conference of the Society of Applied Algology. The meeting will be held January 28 - February 2, 1990 in Tiberias, Israel. Main themes include: technology of algal biomass production, products from algae and their use, genetics and cell biology, environmental limitations and growth physiology, and new technologies (harvesting and reactor design). A conference package is available for US $440 (double occupancy), including registration, room, and most meals. Deadline for abstracts and payment at the package rate is October 15, 1989. Contact: Conference Secretariat, Algology Conference, Melia Te'um, POB 8388, Jerusalem 91082, Israel. (Tel) 972-6-792950, (Telex) 6703 JRTIB, (Fax) 972-2-790453.

The 5th International Symposium on NITROGEN FIXATION WITH NON-LEGUMES will be held in Florence from September 10-14, 1990. The scientific program will include papers invited lectures on the following main topics: (1) Free-living diazotrophs, (2) Root-associated diazotrophs, (3) Nitrogen-fixing photosynthetic microorganisms, and (4) Diazotrophic actinomycetes. Contact: M. Vincenzini - Istituto di Microbiologia Agraria e Tecnica, P.le delle Cascine, 27 - I - 50144 Firenze, ITALY.

POSITIONS AVAILABLE

CONTACT: Florence Gleason, Plant Biology, University of Minnesota, 220 Bio Science Center, 1445 Gortner Ave. St. Paul, MN 55108
RESEARCH: Structure and function of thioredoxin in cyanobacteria, specifically, isolation and investigation of the activity of two thioredoxins from Anabaena.
REQUIREMENTS: Ph.D. in Chemistry, Biochemistry, Microbiology. Experience in protein chemistry, including purification, physical characterization, and kinetic analysis.
SEND: CV and three letters of recommendation.
SALARY: US $18,000 - $20,000 per year.
START: (Expected) July 1, 1989.

CONTACT: Himadri Pakrasi, Dept. of Biology, Box 1137, Washington University, St. Louis, MO 63130, U.S.A. (Tel) 314-889-6853.
RESEARCH: Molecular biology and biochemistry of a membrane-bound photosynthetic protein complex. Conventional mutagenesis and targeted mutagenesis to create novel mutations.
REQUIREMENTS: Experience in molecular biology and/or protein chemistry desirable.
SEND: CV and names of references.
START: Available immediately.

CONTACT: Hans Paerl, University of North Carolina, Institute of Marine Sciences, 3407 Arendell Street, Morehead City, N.C. 28557 (Tel) 919-726-6841.
RESEARCH: Develop and apply immunoassay and DNA hybridization techniques to identify and characterize marine and freshwater nitrogen-fixing microorganisms (bacteria and cyanobacteria).
REQUIREMENTS: Recent Ph.D., with experience in molecular biology, genetics, or immunology. Experience with immunological or hybridization techniques desirable.
SALARY and DURATION: Negotiable.

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BORIS GROMOV has brought to our attention a recent publication from the Academy of Science of the U.S.S.R., entitled "The Microalgae in Collections in the USSR". The monograph (134 pages, in Russian and English) describes eight algal collections in the Soviet Union, and gives addresses for many more outside. While most of the collections are weighted towards eukaryotic algae, this monograph nonetheless provides a rich source of novel cyanobacterial strains. The chapter on the collection of the Biological Institute of Leningrad University alone provides brief descriptions of well over a hundred cyanobacteria.

DON BRYANT tells us of recent progress in his laboratory sequencing cyanobacterial genes encoding components of Photosystem I. The following genes have now been sequenced completely:

- psaC - from Synechococcus PCC 7002, from Nostoc sp. MAC PCC 8009, and from the cyanelle of Cyanophora paradoxa (an honorary cyanobacterium).
- psaD - (ferredoxin-docking protein) from Nostoc sp. MAC PCC 8009.
- psaE - from Synechococcus PCC 6301 and from Nostoc sp. MAC PCC 8009.

In addition, work is in progress for the following genes:

- psaE - from Synechococcus PCC 7002: cloned, not yet sequenced.
- psaF - (cytochrome c-553- and plastocyanin-docking protein) from Synechococcus PCC 7002: sequencing in progress.

NOVEL CYANOBACTERIAL METHYLASE RAISES PHYLOGENETIC QUESTIONS

CHRISTIAAN KARREMAN has published his Ph.D. work, entitled "The genes for two procaryotic DNA-modification methylases". Though not evident from the title, the work is of specific interest to cyanobacteriologists, especially those with an evolutionary bent. One of the two methylases investigated, M.AquI, is found in Synechococcus PCC 7002, and related enzymes are found in several strains of Anabaena and Nostoc. It has been classed as a Type II methylase because its cognate restriction enzyme, R.AquI, cuts at a defined location, unlike Type I and Type III restriction enzymes. C.K.

cloned and sequenced DNA from PCC 7002 encoding this methylase. The amino acid sequence for the predicted protein shares homology with previously determined Type II cytidine-modifying methylases. However, all known Type II methylases are encoded by a single gene, while expression of M.AquI requires two genes. The conserved regions are divided between these two genes. Type I methylases also require expression of two genes, and C.K. speculates that cyanobacteria may have diverged from the line leading to Gram negative and Gram positive bacteria before the appearance of the distinct types of restriction/modification systems that we observe today.

PATTERN OF PROTEIN SYNTHESIS RELATED TO SALT TOLERANCE

SHREE KUMAR APTE reports on recent work he and Arvind Bhagwat completed, aimed at understanding mechanisms underlying salt tolerance in cyanobacteria. They compared patterns of protein synthesis in two strains of Anabaena: strain L-31, a salt-sensitive freshwater strain, and A. tolurosa, a salt-tolerant strain isolated from brackish water. With both strains, conditions of salt stress altered the pattern of protein synthesis, and with both the degree of alteration depended on the salt concentration. Saturation occurred well below the 50% lethal dose of salt. However, the the two strains differed in important respects. With strain L-31, salt-stress-induced proteins appeared only transiently after exposure to high salt, but with A. tolurosa, such proteins persisted throughout the period of stress. Furthermore, the induced proteins of strain L-31 were mostly confined to the cytoplasmic fraction, but those of A. tolurosa were found in significant number in the membrane fraction as well.

ANABAENA AND YEAST GENES WELL MATCHED

Those interested in expressing bacterial genes within yeast might do well to look towards the cyanobacteria, suggest Manjula Mathur and RAKESH TULI. It has been observed that for a given organism, highly expressed genes show a marked bias in codon usage. The better the codon usage of a gene matches that of highly expressed genes, the better it tends to be expressed. One measure of the degree of this correspondence is the Codon Adaption Index (CAI) described by Sharp and Li [(1987) Nucl.Acids Research 15:1281-1295]. Our correspondents used this measure to predict the level of expression of nifHDK genes, encoding nitrogenase, in the yeast Saccharomyces cerevisiae. Of sixteen published sequences for nifH, the gene from Anabaena PCC 7120 had codon usage most like highly expressed yeast genes. The CAI for the cyanobacterial nifH gene is 0.302, while the CAI for nifH from other bacteria fall in the range of 0.067 to 0.164. The CAI for the nifH gene from Klebsiella pneumoniae was only 0.1. This gene is of special interest because the Klebsiella genes have been used by different groups to examine transgenic nif expression in yeast. The story is much the same for nifD and nifK: the genes from Anabaena have much higher CAI's (0.244 and 0.289) compared to their counterparts from Klebsiella (0.07 and 0.066).

CLOSED PHOTOBIOREACTOR SHOWS ADVANTAGES IN CYANOBACTERIAL GROWTH

CyanoNews recently sponsored a panel discussion on the mass culture of Spirulina. One theme that was reiterated throughout the discussion was the need for improved reactor design, in particular, those making use of closed systems. JOHN BENEMANN sent us a recent report of work he did with K. Miyamoto and O. Wable [Biotech. Lett. 10:703-708] describing a closed photobioreactor that appears to have several advantages over older designs in small scale microalgae production. Vertical tubular reactors (VTRs) were constructed from commercially available glass tubes that are mass produced for the fluorescent light bulb industry. A 5 cm (inner diameter) by 2.35 m tube costs $1.50 and can be adapted to support growth of a 4 liter culture. CO_2-enriched air was sparged in at the bottom of the tubes, and air escaped from the top. Five cyanobacterial species (Anabaena sp., Nostoc 29106, Tolypothrix sp., Anacystis sp., and Chloroploeopsis sp.) were tested, as well as species of unicellular diatoms and green algae.

In addition to their low cost, VTRs exhibit several other advantages: (1) the scouring action of bubbles prevent wall growth even after prolonged cultivation, (2) high CO_2 utilization efficiency can be achieved simultaneously with high productivity (this is of particular importance in applications such as the production of isotopically labeled carbon compounds), (3) build-up of oxygen is avoided, (4) productivity is relatively high, and (5) the system is simple and easy to operate.

DNA REPLICATION IN CYANOBACTERIA AND CHLOROPLASTS COMPARED

We cyanobacteriologists feel a close kinship with those who study chloroplasts, considering them (chloroplasts) to be cyanobacteria that somewhere took a wrong turn. HENRY DANIELL tells us of his attempt to bridge the evolutionary gap by returning an origin of DNA replication from a chloroplast to a cyanobacterial relative. He has constructed a plasmid, pHD407, that carries one of the two unidirec-

tional origins from pea chloroplast on a 4.1 Kb fragment [Meaker et al. (1988), Mol Cel Biol 8:1216-1223]. The plasmid did indeed transform Synechocystis PCC 6803 to chloramphenicol resistence, but it has not been detected in a free-living state. Since the 4.1 Kb fragment also contains portions of the highly conserved genes encoding 16S and 23S rRNA, it would not be surprising if pHD407 had simply integrated into the chromosome by homologous recombination.

Two in vitro systems were used to compare the initiation of DNA replication at the chloroplast origin to that at the origins of the three endogenous plasmids of Synechocystis. Extract from pea chloroplast supported DNA synthesis initiated from the chloroplast origin, but not from Synechocystis DNA. Conversely, extract from Synechocystis supported DNA synthesis initiated from Synechocystis DNA but not from the chloroplast origin. Both systems failed to initiate DNA synthesis from the origin of pBR322. H.D. intends to use these in vitro systems to determine the minimal DNA sequences required to support initiation.

CONDITIONAL-LETHAL GENE TO SELECT FOR GENE REPLACEMENT IN ANABAENA

Directed gene replacement has long been routine with several unicellular cyanobacteria. It has proven more difficult to replace genes in Anabaena, in part because plasmid DNA introduced by conjugation generally inserts into the chromosome by single recombination, leading to gene duplication, not gene replacement. YUPING CAI has found a simple, effective means of selecting for rare double recombinants. The technique employs the sacB gene from Bacillus subtilis, encoding a secreted levansucrase. When this gene is introduced into Anabaena PCC 7120, the strain becomes sensitive to 5% sucrose in solid media (but grows perfectly well in the absence of sucrose). The sacB gene was inserted into the vector portions of plasmids carrying either an insertionally inactivated nifD gene (encoding a component of nitrogenase) or hetA gene (required for the differentiation of functional heterocysts). The plasmids were conjugated into Anabaena, and almost all drug-resistant, sucrose-resistant colonies proved to manifest the phenotype expected from gene replacement. Gene replacement was confirmed by Southern hybridization analysis.

In testing the conditional lethality of sacB in Anabaena, a sucrose-resistant strain was recovered in which the sacB gene was interrupted by a foreign sequence. This 1.7-kb sequence is found in several copies in the genome of Anabaena PCC 7120 and appears to function as an insertion sequence.

GENETIC MANIPULATION OF A FULLY HETEROTROPHIC CYANOBACTERIUM

Genetic manipulation of photoheterotrophic cyanobacteria has provided important insights into the molecular workings of Photosystem II. Mindful of this, many laboratories have considered applying the same bag of tricks to study Photosystem I. Unfortunately, there has been no report of any transformation or conjugation of a fully heterotrophic cyanobacterium. IRIS MALDENER sends us the welcome news that she has succeded in introducing DNA into the chromosome of the heterotrophic Anabaena ATCC 29413 (strain FD). Working in collaboration with Wolfgang Lockau and Peter Wolk, she isolated a 5-kb DNA fragment containing a gene from Anabaena ATCC 29413 that encodes a calcium-dependent protease. A cartridge specifying resistance to kanamycin was inserted into this gene, and the resulting plasmid (also containing sacB -- see the previous report) was conjugated into Anabaena ATCC 29413 (strain FD), yielding a large number of kanamycin-resistant colonies. One colony, arbitrarily chosen, was purified and plated on medium containing sucrose to select for double recombinants. All seven colonies that were analyzed by Southern hybridization showed patterns expected from gene replacement. I.M. is now characterizing the mutant, with particular regard to its ability to differentiate heterocysts.

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